J. Chem. & Cheml. Sci. Vol.4 (1), 51-57 (2014)

Neuromedin Peptide Analogue Attached Carbon Nanotubes for Triggering Intercellular Pathways

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(Received on: January 14, 2014)

ABSTRACT

Synthesis and functionalization of Single walled carbon nanotubes (SWNT) with neuromedin peptide analogue which has a promising application in triggering the intracellular path ways was successfully achieved and characterized. Neuromedin peptide analogue were synthesized by the modified Fmoc solid phase peptide synthetic strategy by using Polyethylene glycol acrylamide (PEGA) as a solid support. Carbon nanotubes are functionalized by a peptide (neuromedin analogue) via diimide-activated amidation under ambient conditions. The nanotube-Neuromedine conjugates thus obtained were characterized using FT-IR, MALDI-TOF-MS, Raman spectra and EDAX.

Keywords: SWNT, Neuromedin, Fmoc, Polyethylene glycol acrylamide, Carbon Nanotubes, FT-IR, MALDI-TOF-MS.

1. INTRODUCTION

Single walled carbon nanotubes[1] (SWNT) play an important role in the emerging field of biological nanotechnology. In the field of health care, CNTs have recently gained popularity as a promising potential drug carrier which will help to solve the problems like inefficient distribution, lack of selectivity etc during the administration of drugs[3].

Here our research mainly focuses biologically active neuromedin peptide attached with functionalized SWNT, which has a promising application in triggering the intracellular path ways. The clear structure (Fig1) shown here for better understanding.

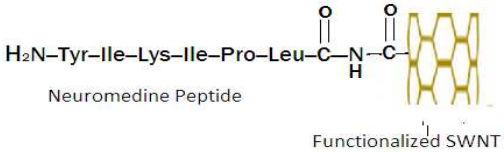


Fig. 1. Functionalized SWNT with neuromedin peptide analogue

The main advantage of using SWNT as a drug carrier or with a biosensor compared to free drug is the potential to target delivery for selective distribution of certain types of cells, reducing the toxicity to non targeted cells3. Biomolecules can be immobilized on SWNT for potential biosensor systems. The Functionalisation of SWNT with biomolecules is a relatively new direction in exploring the chemistry of SWNTs to be the basis of new diagnostic tools, anti-cancer treatment, gene therapies and vaccines[4]. The present work focus on synthesis and functionalization of SWNT with neuromedin peptide analogue which has a promising application in triggering the intracellular path ways[1,3&6]. Neuromedin peptide analogue was synthesized by the solid phase organic synthetic strategy using PEGA solid support[7,8]. Carbon nanotubes are functionalized by peptide (neuromedin analogue) via diimide-activated amidation under ambient conditions[5]. The nanotube-Neuromedine conjugates thus obtained are characterized using FT-IR, MALDI-TOF-MS, Atomic force microscopy (AFM) and Thermal gravimetric analysis show that the conjugate samples indeed contain both carbon nanotubes and neuromedin and that the peptide species are intimately associated with the nanotubes[2,6].

2. EXPERIMENTAL

2.1 Materials

Fe_2O_3, fumed-alumina nano particles, $Fe(NO)_3 9H_2O$, Methanol, where purchased from Merk India and Alfa-easer England, Argon, methane Polyethyleneglycol amine resin(100-200mesh), Fmoc-Tyr(trt)-OH, Fmoc-Ile-OH, Fmoc-Lys(boc)-OH, Fmoc-Pro-OH,Fmoc-Leu-OH, Dichloromethane (DCM), Dimethylformamide (DMF), Methanol, N-methyl-2-pyrolidone(NMP), Dicyclopropylecarbodiimide (DCC), Diiso-propylethylamine (DIPEA), 4Dimethylamin-opyridine (DMAP), Hydroxzybenzotiazole (HOBt), Thioanisole, EDT, Diisopropy-lcarbodiimide (DIC), Trifluroaceticacid (TFA), Triisopropylsilane (TIS), Aceticacid, Pyridine, Pipyridine and diethylether etc.

All the above amino acids and chemicals were purchased from Sigma-Aldrich, Germany , China and Switzerland, Alfa-asear England, Novabiochem Germany, E. Merk (India) and lobachemi Mumbai which the highest purity available and the purity of the amino acid was tested and the solvents are degassed when required and all the reagents are stored and used in proper way.

2.2 Synthesis of SWNT using methane CVD method

Supported catalyst, Fe_2O_3 catalyst is prepared by impregnating 1g of fumed-alumina nano particles with 30ml of a methanol solution that contains 0.245g of $Fe(NO)_3.9H_2O$. The impregnation typically lasts for 1hr at room temperature. The methanol solution was then removed via rotary evaporation at 80^0C. The material is then heated at 150^0C overnight followed by grinding into a fine powder. This resulting catalyst is denoted as Fe_2O_3/alumina[2]. For a methane CVD experiment, 10mg of the catalyst was placed in a quartz tube mounted in a tube furnace. An argon flow was passed through the quartz tube as the furnace was heated to reach 1000^0C. The Argon flow was then replaced by methane (99% purity) at a flow rate of 6150 cm^3rm in under 1.25atm.

head pressure. The methane flow lasted for 10min and was replaced by argon and the furnace was cooled to room temperature[2,5]. The sheet form of SWNT were obtained, the SWNT was collected and characterized and Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) analysis were done. The clear analytical reports are given in (Fig 3&4).

2.3 Synthesis of neuromedin (NH_2-Tyr-Ile-Lys-Ile-Pro-Leu-COOH) (Modified Fmoc solid phase peptide synthetic strategy)

PEG amine resin(150mg) were transferred to well clean sililated and sterilized peptide synthesizer, to that sufficient amount of N-methyl Pyrolidone were added and swelled for 1hr and removed the filtrate under vacuum. Fmoc group from resin were removed and washed[8.] Fmoc amino acid dissolved in minimum quantity of NMP in well closed 25ml RB flask to that HOBt were added and dissolved and DIC were added and shaked it well for 3mins and immediately the content was transferred in to the resin with moisture free atmosphere and shaked it well for 5mins, to that DIPEA were added and shaked well for 45mins. Reaction progress was monitored by TLC. Small pinch of the resin were taken and washed, ninhydrin were tested in case positive means same amino acid coupling was repeated, in case negative means washed and deprotected[8,9]. The remaining amino acids coupling were done by above method.

2.3.1Cleavage of crude peptide from resin

After synthesis the resin was washed with hexane, DCM, $CHCl_3$ and MeOH and dried. The cleavage was done with 95%TFA:2.5%TIS:2%water:0.5%m-crosal at 3hours under nitrogen atmosphere and the resin was washed 4times with TFA, the filtrate were collected in 50ml RB-Flash and all the traces of TFA was evaporated by using Rota vacuum evaporator[7,9]. The peptide was isolated with excess of peroxide free pure cold diethylether and the peptide was washed 7 times with diethylether and centrifuged. The clear white powder form of peptide was collected in a small tubes and sealed[9].

2.3.2 Purification and Analysis of the peptide

The crude peptide purity was tested by using HPLC – High Performance Liquid Chromatography[8]. The all impurity was removed and the expected peptide were extracted by RP-Preparative HPLC and lyophilized and the peptide was allowed to test with MALDI TOF-MS Spectroscopy.

2.4 Functionalization of SWNT with neuromedin via diimide- activated amidation

In a typical experiment for the amidation reaction with Neuromedin peptide, a SWNT sample and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) were added to an aqueous KH_2PO_4 buffer solution. After the mixture was sonicated for 2 h, neuromedin was added. The resulting mixture was stirred at room temperature for 24hrs and then transferred to a membrane tubing (cut off molecular weight 12000) for dialysis against freshwater[1,6]. Following the dialysis for 3 days, the mixture was vigorously centrifuged at a high speed

(7800 rpm) to separate the insoluble nanotubes, yielding a homogeneous solution[4]. Free Neuromedin in the solution was removed via dialysis again in a membrane tubing of a larger pore size (cut-off molecular weight 100000) for 3 days. Upon the evaporation of water, a solid-state SWNT- Neuromedin conjugate sample was obtained[5,6]. The conformation analysis is discussed and TEM characteristic analysis reports are shown in (Fig4).

3. RESULT AND DISCUSSION

Neuromedin Peptide was successfully synthesized using solid phase peptide chemistry (Fmoc chemistry) and purified using HPLC. The desired peptide was identified using MALDI TOF MS.

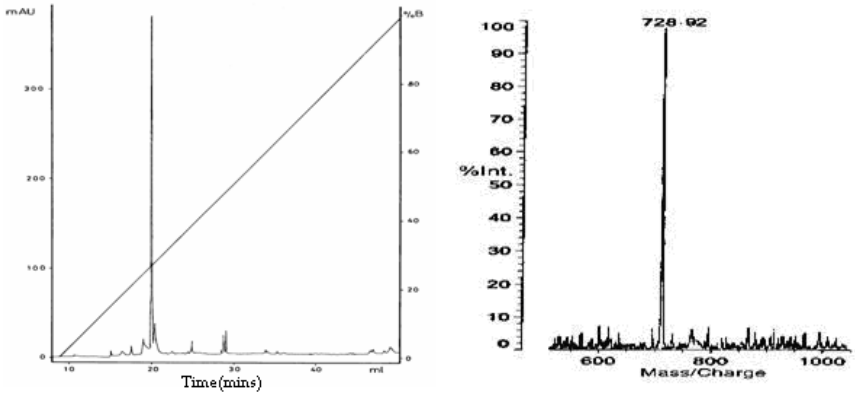


Fig 2 : HPLC profile and MALDI TOF MS of Neuromedin

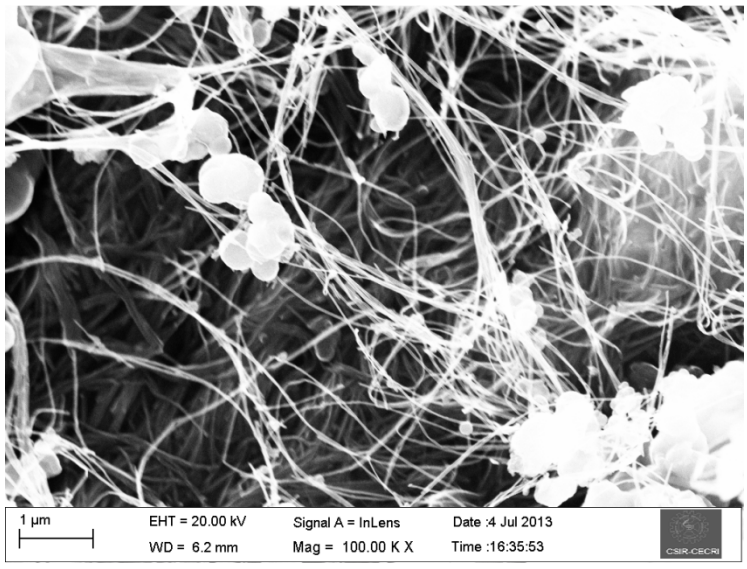


Fig 3 : SEM image of SWNT

Synthesized Single walled carbon nanotubes (SWNT) analogue were characterized by standard SEM analyzer the characteristic image was given here.

Functionalized Single walled carbon nanotubes (SWNT) with neuromedin peptide analogue were successfully achieved and characterized by TEM analyzer, the characteristic analysis report were given here.

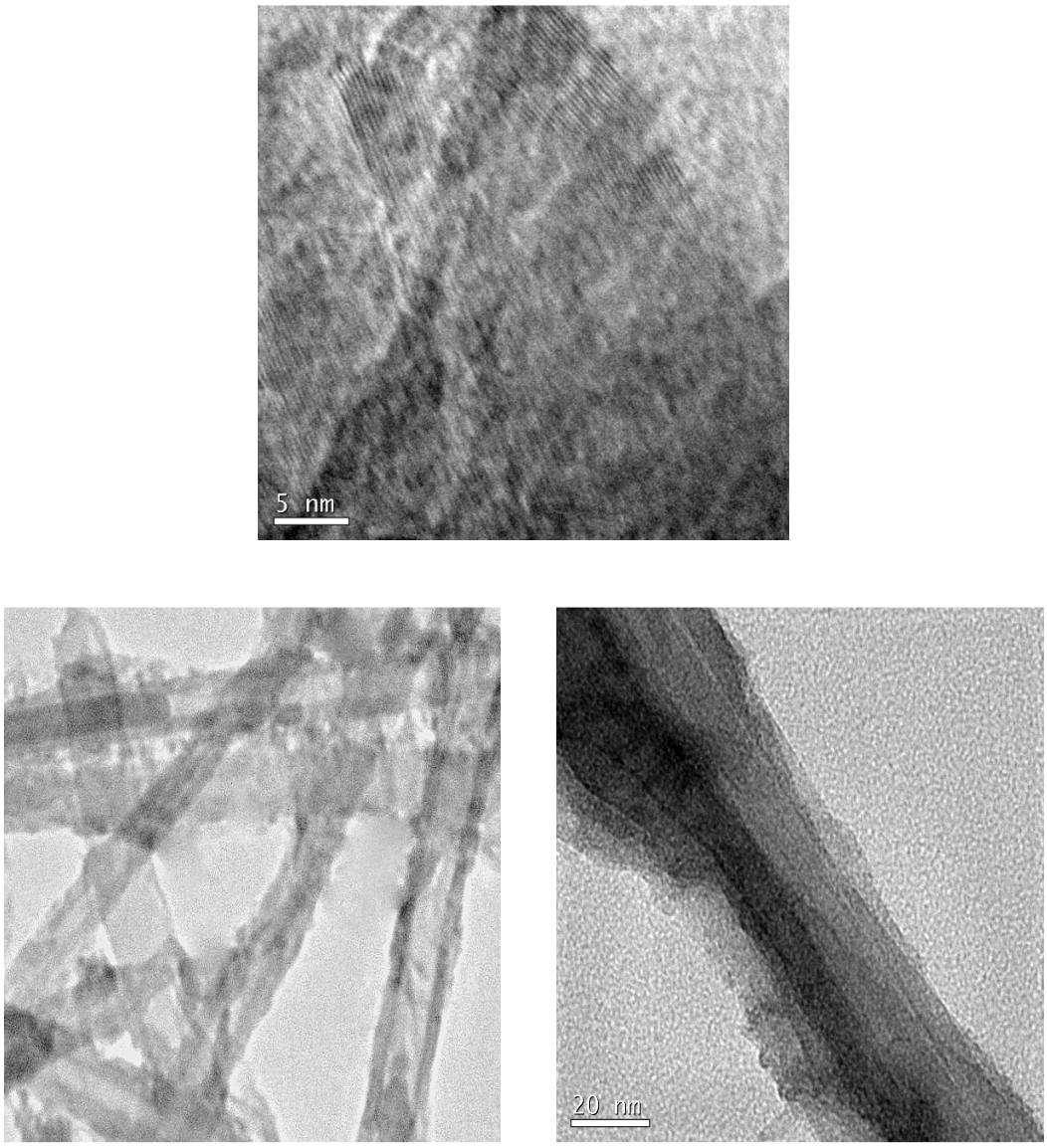


Fig 4 : Transmission electron microscope (TEM) images of CNT network attached with Peptide template

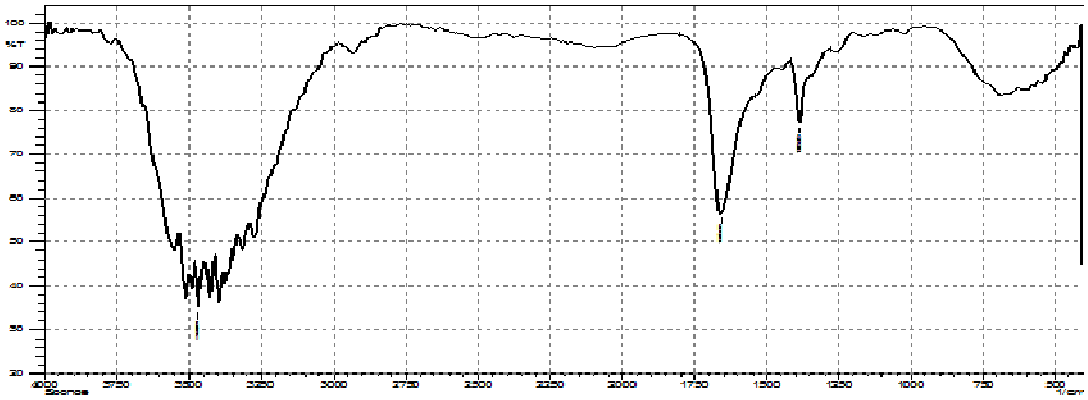
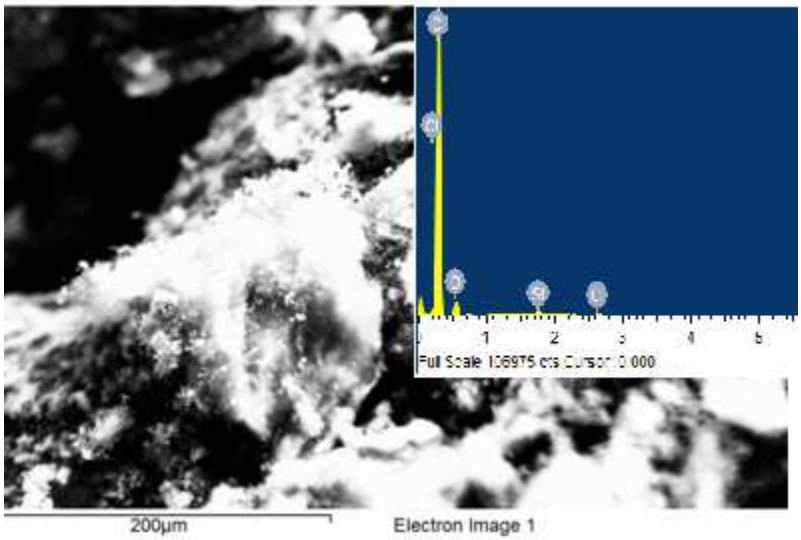


Fig.5. FT-IR spectra of functionalized CNT



Element	Weight%	Atomic%	Compd%	Formula
C K	27.26	33.31	99.88	CO2
Si K	0.05	0.03	0.10	SiO2
Cl K	0.02	0.01	0.00	
O	72.67	66.66		
Totals	100.00			

Fig. 6: Edax analysis of SWNT

Neuromedine-SWNTs are character-rised using FT-IR Spectroscopy, Raman Spectroscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The clear MALDI TOF-MS and TEM analysis report shows our successful achievement of Single walled carbon nanotubes (SWNT) with neuromedin

peptide analogue which has a promising application in triggering the intracellular path ways.

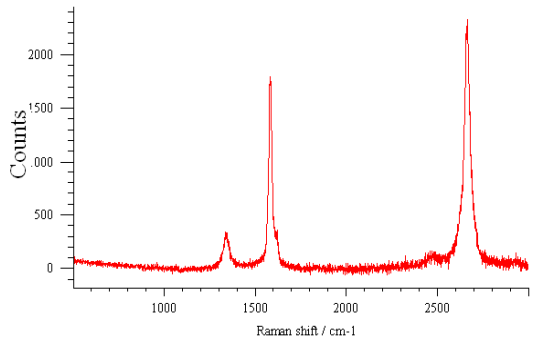


Fig.7 Raman spectra of Functionalized SWNT

4. CONCLUSIONS

Here demonstrated a successful approach of using peptides to attach on SWNT for biomedical applications. Surface functionalization chemistry is the most essential and fundamental part for CNT biomedical applications. Their application will lead to the design of ultrasensitive biosensors and diagnostic tools for medical, environmental and security applications. CNTs, especially SWNTs are highly promising in biomedicine due to several features. CNTs are composed purely of carbon, while many inorganic nanomaterials (e.g. quantum dots) are composed of relatively more hazardous elements, such as heavy metals. Neuromedin-CNT conjugate can act as biosensor for triggering the intracellular path ways.

ACKNOWLEDGMENTS

This work was supported by Department of Science and Technology, Govt. of India under WOS-A Scheme. I acknowledge Dr. Roice Micheal and Dr. C.P Vinod, Cardiff University, Cardiff, UK, Dr.C Arunan, KSCSTE for their support and guidance.

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